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EFFECTS OF MALATHION AND DIAZINON EXPOSURE ON FEMALE GERMAN COCKROACHES (DICTYOPTERA: BLATTELLIDAE) AND THEIR OOTHECAE

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Abstract.—Female German cockroaches, *Blattella germanica* (L.), were exposed to malathion and diazonon by tarsal contact. The effects of exposure on hatch of oothecae and on newly-emerged nymphs are compared in resistant and susceptible strains. Insecticide exposure increased the frequency of oothecal drop over that which occurred naturally in the susceptible but not in the malathion or diazinon-resistant strains. The time from exposure to hatch was decreased when oothecae hatched on a treated surface in all except oothecae of the diazinon-resistant strain. Exposure to diazinon, but not malathion, decreased nymphal emergence. Nymphs of the susceptible strain that emerged on treated surfaces were unable to shed the embryonic cuticle, whereas 18 to 23% of resistant strain nymphs were upright and moving freely. Results are compared to similar experiments with propoxur.

Key Words: Insecticides, behavior

Many studies of the German cockroach, *Blattella germanica* (L.), have dealt with insecticide resistance. Behavioral responses to insecticides have received less attention. German cockroaches are repelled by certain insecticides (Ebeling et al. 1967, 1968, Ebeling et al. 1966), but little is known concerning possible modifications of the repellency response following several decades of selection pressure from insecticides. Bret and Ross (1985, 1986) reported evidence that behavioral modifications have indeed accompanied the development of insecticide resistance. Dispersal and grooming behavior in adults of a susceptible strain exposed to propoxur vapors differed from that of adults in a propoxur-resistant field strain.

Irritability and repellency are widely recognized responses to insecticides (Lockwood et al. 1984), but in *B. germanica*, a third response has been reported. Insecticide exposure may cause a female to drop her ootheca prematurely (Woodbury 1938, Parker and Campbell 1940, Russell and Frishman 1965, van den Heuvel and Shenker 1965, Chadwick and Evans 1973, Muller and Coch 1975, Barson and Renn 1983, Harmon and Ross 1987). In two of the foregoing studies, comparisons were made between resistant and susceptible strains. Russell and Frishman (1965) reported a higher frequency of premature drop among susceptible than chlordane-resistant females when exposed to vapors from dichlorvos resin strips. The ages of the oothecae were unknown. Harmon and Ross (1987) also found a higher retention of oothecae by resistant than susceptible females. They used females of a propoxur-resistant field strain and a laboratory susceptible strain (VPI) that carried oothecae in Stage XII of embryonic development (Tanaka 1976), that is, oothecae that were due to hatch within 48 to 72 h after selection.

When oothecae are within 2 to 3 days of hatch and conditions approximate those typical of German cockroach infestations (room temperature; r.h. usually above 30%), hatch and nymphal survival depend primarily on whether the ootheca falls on a treated or untreated surface. Van den Heuvel and Shenker (1965), Barson and Renn (1983) and Harmon and Ross (1987) found treatment of females bearing mature oothecae had little effect on hatch or productivity of the ootheca unless it dropped on a treated surface. On the other hand, when oothecae hatched on a treated surface, nymphal emergence and survival, when studied, were affected. Decreases in the latter effects due to hatch on a propoxur-treated surface were less in a propoxur-resistant than in a susceptible strain (Harmon and Ross 1987).

Reported here are experiments on the effects of malathion and diazinon on females carrying mature oothecae. The purpose was two-fold: first, to compare the effects on females of resistant strains to those of susceptible strains; secondly, to compare the effects of two organophosphates (malathion and diazinon) to those of propoxur, a carbamate.

Materials and Methods

Ootheca-bearing females were from three strains: the VPI strain, our standard susceptible laboratory strain; the Carver strain, a field strain resistant to malathion, propoxur, and pyrethrins, collected in Gainesville, Fla., in 1983 (Cochran, pers. commun.); and the Lynn Haven strain, a diazinon-resistant strain collected in Lynn Haven, Va., in 1983 (Cochran, pers. commun.).

Cockroach rearing conditions and test procedures were like those of the propoxur experiments (Harmon and Ross 1987). In brief, females carrying 20–24 day-old first oothecae were lightly anesthetized with CO_2 and examined under low power of a binocular microscope to determine the stage of egg development, using Tanaka's (1976) developmental table. Females carrying Stage XII oothecae were selected for test purposes, that is, females carrying oothecae due to hatch within 48 to 72 h. Only those oothecae with normal-appearing embryos in each compartment or, at most, no more than three undeveloped eggs, were used. The females were held for 24 h and then exposed by tarsal contact to filter paper treated with either malathion or diazinon (Cochran 1973). The filter paper (Whatman #1) was cut in 15 × 15 cm squares, placed on a glass plate, and impregnated evenly with a 3 ml mixture of risella oil and trichloroethylene (1:2 v/v) and either 0.08 ml of technical grade malathion (0.356 $\mu l/cm^2$) or 0.015 ml of technical grade diazinon (0.067 $\mu l/cm^2$). Dosages were selected by preliminary experimentation so as to give an approximate mortality of 65% among VPI strain females following an exposure period of 100 min. The purpose was to make the results comparable to prior work with propoxur, where 65% of the VPI strain females died as a result of a 100 min exposure to propoxur-treated filter paper (Harmon and Ross) 1987).

Groups of 10 to 12 ootheca-bearing females were confined by a glass chimney on the treated papers or on unused filter paper (controls) after the papers had dried for 1 h. Initial tests with the TCE/risella oil mixture showed that, as expected, neither lethality nor oothecal drop were affected. TCE evaporates rapidly and the possibility that risella oil, basically a mineral oil, would affect the cockroaches was highly unlikely. The experiments were begun between 9:00 and 11:00 h. Oothecal drop during exposure was recorded. Following exposure, females that dropped oothecae were separated from those that retained oothecae. The latter were placed in separate jars for subsequent observations, as follow: retention of oothecae at 24 h after exposure; hatch of oothecae; time from the end of the exposure period to hatch; number of embryos remaining in the ootheca; number of nymphs that emerged; and the number that were alive and whether they were upright and/or fully pigmented 24 h after hatch. Like observations were made on oothecae that dropped during the exposure period. They were kept on the treated papers but were placed in individual jars with a water source 5 to 7 cm above the bottom of the jar that maintained a high humidity (85–90% r.h.) within the jar, ensuring the oothecae did not dry out. The purpose was to assess the effects of a treated surface on hatch and nymphal survival. Additional data were obtained by manually detaching oothecae of like stage to those carried by females at the time of exposure and placing them in individual jars on treated papers as described above or, in the case of the controls, on clean filter paper. Female mortality was recorded at 72 h after exposure. At that time, mortality was complete and could be compared to that in the propoxur experiment.

Percentages of oothecal hatch, nymphal emergence, nymphal survival, and freely-moving nymphs were calculated. Nymphs were counted as live if pulsation was occurring in the dorsal blood vessel. This included nymphs that were alive, but that were unable to free themselves from the embryonic cuticle. Percentage survival was based on the number of live nymphs among those that emerged successfully in order to distinguish between death that occurred within the oothecae (reduced emergence) from that due to emergence on a treated surface.

Data were analyzed using the Statistical Analysis Service (SAS) and the Bio-medical Data Processing (BMDP) statistical software available on the VPI & SU IBM mainframe computer. The best statistical model to analyze each type of data was determined by loglinear models (BMDP). Percentage premature drop of oothecae was analyzed by a least significant differences (LSD) pairwise comparison procedure. The association between mortality and premature drop was analyzed by establishing two-way frequency tables. The null hypothesis was that post treatment mortality among females that retained their oothecae did not differ significantly from that of females that dropped their oothecae. Percentage hatch was analyzed using a one-way ANOVA ($F = 19.79$; $df = 1075$; and $P > 0.0001$) followed by a LSD comparison test. Times from exposure to hatch were analyzed using a general linear models procedure ($F = 134.55$; $df = 893$; and $P > 0.0001$) followed by a LSD comparison test. Means were calculated for each treatment group and sorted in ascending order. Significance groupings were assigned from LSD tests. Estimates of percentage nymphal emergence, survival, and freedom of movement were arc-sine transformed and then analyzed as above with a general linear models procedure and LSD pairwise comparison.

Results

None of the VPI strain females died during exposure to untreated filter paper and, in the two resistant field strains, only one (3%) died in each strain (Table 1). Mortality due to malathion exposure of VPI strain females did not differ significantly from that due to diazinon exposure, as expected due to pre-selection of dosages. Resistance in the Carver strain reduced mortality from malathion to approximately half that of the VPI strain. No mortality occurred following exposure of Lynn Haven strain females to diazinon.

Premature drop of oothecae during the exposure period occurred only in the malathion experiment. It was limited to 3 oothecae in one strain and 4 in the other (Table 1). No oothecae were dropped by the control females or those in the experiment with diazinon. A tendency towards higher mortality of females that dropped oothecae than of those that retained oothecae was evident. Mortality was significantly higher among the

Table 1. Effects of malathion and diazinon on mortality and oothecal drop in female *B. germanica* carrying Stage XII: oothecae (oot).[a]

Strain	No. of ♀♀	% Mortality[c]			oot Drop during Exposure		oot Drop 24 h after Exposure	
		All ♀♀	♀♀ Dropped oot during Exposure	Retained oot	No.	%	No.	%
Control Data[b]								
VPI	27	0 a	—	0 a	0	0 a	4	15 bc
Carver	34	3 a	—	3 a	0	0 a	7	21 c
Lynn Haven	30	3 a	—	3 a	0	0 a	5	17 bc
Malathion Treatment								
VPI	100	56 c	66 b	55 c	3	3 a	28	28 c
Carver	102	24 b	50 a[d]	23 b	4	4 a	17	17 bc
Diazinon Treatment								
VPI	100	63 c	—	63 c	0	0	26	26 c
Lynn Haven	48	0 a	—	0 a	0	0	4	8 a

[a] Means within a column followed by the same letter are not significantly different ($P > 0.05$).
[b] Control females were exposed to untreated filter paper.
[c] 72 h after exposure to untreated or treated filter paper.
[d] Significantly higher than mortality of females that retained their oothecae (23%) ($P > 0.05$).

former when results from the two strains were pooled ($P > 0.05$).

Normal drop and hatch of oothecae was expected to occur in the period from 48 to 72 h after selection. Oothecae 24 h after exposure were aged 48 h plus 100 min (time of exposure of females to malathion, diazinon, or clean filter paper). At this time, control females had begun to drop their oothecae. The frequency of drop by control females ranged from 15 to 21% in the three strains (Table 1). Percentage drop by VPI strain females was increased significantly over that of unexposed females when the females had been exposed to either malathion or diazinon. The difference between naturally occurring drop and that found among insecticide-exposed VPI strain females gives a rough estimate of drop due to the insecticide, i.e. 13% were dropped prematurely due to malathion and 11% due to diazinon. In the Carver strain, drop following exposure to malathion did not differ significantly from that in the controls. Retention of oothecae by Lynn Haven strain females exposed to diazinon was significantly longer than that by control females.

Table 2 summarizes the effects of malathion and diazinon on hatch of oothecae and on the newly-hatched nymphs. "Days to hatch" refers to the time between the end of the exposure period and hatch (opening) of the ootheca. In the control data, results were closely similar. The only notable variations were that detached oothecae of VPI strain females took longer to hatch than those that hatched naturally (attached) and hatch time of the latter was significantly less than in the Carver and Lynn Haven strains.

In the malathion experiment, VPI and Carver strain oothecae hatched more quickly on the treated surface (oothecae dropped or detached) than on an isecticide free surface (Table 1, control data and oothecae attached to malathion-exposed females). Hatch time was also decreased when VPI strain oothecae hatched on a diazinon treated surface.In one instance only, the time required for hatch on a treated surface was not decreased over that in the control data. That was among detached Lynn Haven strain oothecae.

In general, the number of oothecae that hatched (opened to permit nymphal emer-

Table 2. Effects of malathion and diazinon on hatch of Stage XII *B. germanica* oothecae (oot) and on nymphal emergence and survival.[a]

Strain	oot	Days to Hatch[b]	oot Hatched n	oot Hatched %	Nymphs from Hatched oot % Emergence	% Survival[c]	% Freely Moving[d]
			Control Data				
VPI	attached	1.9 b	27	100 a	96 a	100 a	100 a
	detached	2.1 c	36	92 a	95 a	100 a	100 a
Carver	attached	2.7 cd	32	100 a	96 a	100 a	100 a
	detached	2.3 c	29	85 b	90 b	100 a	100 a
Lynn Haven	attached	2.5 c	30	100 a	95 a	99 a	100 a
	detached	2.2 c	30	100 a	89 b	100 a	100 a
			Malathion Treatment				
VPI	attached	1.9 b	84	86 b	94 a	100 a	100 a
	dropped	0.3 a	3	100 a	92 a	97 a	2 d
	detached	1.5 b	101	98 a	90 b	94 b	0 d
Carver	attached	2.3 c	95	94 a	91 ab	100 a	100 a
	dropped	1.0 a	1	100 a	93 a	100 a	54 b
	detached	1.8 b	89	94 a	83 c	95 ab	23 c
			Diazinon Treatment				
VPI	attached	3.1 d	80	80 b	83 c	100 a	100 a
	dropped	—	0	—	—	—	—
	detached	1.5 b	102	97 a	83 c	87 b	0 d
Lynn Haven	attached	2.5 c	40	85 b	80 c	100 a	100 a
	dropped	—	1				
	detached	2.3 c	46	93 a	89 bc	99 a	18 c

[a] Means within a column followed by the same letter are not significantly different ($P > 0.05$).
[b] Days from end of exposure period (24 h and 100 min after oot stage identification) to hatch.
[c] % of emerged nymphs that were alive 24 h after emergence.
[d] % of emerged nymphs that were free of the embryonic cuticle and moving about freely 24 h after emergence.

gence) was not decreased due to manual detachment, except for possible injury to a few in the Carver strain (Table 2). In the experimental groups, small but significant reductions occurred among oothecae retained by malation-treated VPI strain females and by diazinon-treated VPI and Lynn Haven strain females (attached oothecae). Some of the VPI and a few of the Carver strain oothecae were from dead females, but death had no perceptible effect on hatch. Once the nymphs had forced open the oothecae at the time of hatch, most emerged successfully. At least 95% emerged from oothecae of the untreated control females, except for 90 and 89% from detached Carver and Lynn Haven oothecae, respectively. Handling of the latter may have injured a few of the eggs. A tendency towards reduced emergence was evident in the malathion experiment, but it was only in the diazinon experiment that treatment had a clear effect on emergence. It was reduced among attached and detached oothecae of both the VPI and Lynn Haven strains.

Nymphs were scored as living at 24 h after exposure of the parent females to untreated or treated filter paper if pulsation was observed in the dorsal blood vessel. On this basis, survival was below 99% only in situations where nymphs emerged on a treated surface (Table 2, dropped or detached). Slight but significant reductions occurred among detached oothecae of VPI and Carver strain females exposed to malathion, although the results were similar for the two strains (94 and 95% survival, respectively). The greatest decrease (87% survival) was among VPI strain nymphs that hatched on a diazinon-treated surface. Survival of Lynn Haven strain nymphs was not affected. Dead nymphs, as well as survivors, were fully pigmented.

The main difference between the effects of malathion and diazinon on newly-hatched nymphs of susceptible and resistant strains was in the ability of nymphs that hatched on a treated surface to free themselves from the embryonic cuticle (Table 2). These nymphs were still alive at 24 h after emergence. Entanglement of VPI strain nymphs on the malathion and diazinon treated surfaces was complete except for 2% of those that emerged from oothecae that dropped on a malathion-treated surface. The percentage of Carver strain and Lynn Haven strain nymphs that freed themselves was significantly higher than in the VPI strain. The nymphs of the resistant strains were upright and could have escaped the treated surface if permitted to do so.

Discussion

Estimates of times between exposure of females to untreated filter paper and hatch may have been affected by slight differences in oothecal development at the time of selection. Nevertheless, it was apparent that oothecae carried by field strain females took longer to hatch than those of the VPI strain. This difference may have been related to either laboratory culture or modifications of resistant field strains. Although Muller and Coch (1975) found that manual detachment increased hatch time, a similar phenomenon was observed here only in the VPI strain.

Hatch on a treated surface decreased hatch time in all experiments except that with the Lynn Haven strain. Another difference in this strain was that diazinon treatment caused a longer-than-normal retention of oothecae. More extensive studies on field strains are needed before it can be determined whether these special characteristics are related to the development of diazinon resistance.

The only effect of insecticide exposure that could be attributed to exposing females rather than to hatch on a treated surface was a slight reduction in the number of oothecae that hatched. Only the Carver strain females did not show this effect. Perhaps it was only when oothecae were retained that there was sufficient time for toxic substances to be passed from the females to the developing embryos.

Emergence was reduced slightly when oothecae hatched on a treated surface. Diazinon had a stronger effect than malathion, especially in the susceptible strain. Possibly vapors from diazinon penetrated the ootheca more deeply than those of malathion, both during exposure of ootheca-bearing females and when oothecae were dropped or placed on a diazinon-treated surface. Lawson (1949) found that diazinon was the only one of several insecticides tested that caused a reduction in emergence.

A basis was laid for comparing the effects of propoxur (Harmon and Ross 1987) to those of two organophosphates, malathion and diazinon, by selecting dosages that caused similar mortality in VPI strain females during the same period (100 min). The primary difference was that premature drop of oothecae due to propoxur was more immediate than that due to the two organophosphates. Delay may have been due to the slower action of malathion and diazinon than of propoxur (Matsumura 1985). Oothecae dropped due to exposure to propoxur would be more likely to fall on a treated surface, as observed in shipboard experiments (Ross and Bret 1986). Whether oothecae would indeed be dropped prematurely depends on whether or not the strain was resistant. A second difference between the effects of propoxur and those reported here was that nymphs that hatched on a propoxur-treated surface died before pigmentation was complete, whereas dead nymphs in the present experiments were fully pigmented. This difference may also be attributed to a slower action of the two organophosphates than of propoxur.

The results of the experiments with malathion and diazinon, considered in conjunction with the earlier study on propoxur, indicate clearly that premature drop of oothecae is a phenomenon associated with susceptibility. It is decreased or, indeed, may disappear entirely among females resistant to chlordane (Russell and Frishman 1965), propoxur, malathion, and diazinon. In addition, Lawson (1949) found a higher mortality among females that dropped oothecae prematurely than among those that retained them and Muller and Coch (1975) reported that premature drop only occurred when the dosage of an insecticide was sufficient to cause the knock-down condition. Mortality and knockdown are, of course, indications of susceptibility. The relative frequency of premature drop by females of a resistant strain compared to those of a susceptible strain may well provide an indication of the frequency of genetically-susceptible females present in the resisistant strain, providing it is not completely homozygous for resistance-conferring genes. Perhaps most insecticides, regardless of differences in mechanisms of resistance, affect the nervous system of susceptible females in a way that leads to premature drop. In any case, the ability of resistant strain females to retain their oothecae following insecticide exposure is clearly an advantageous characteristic. When females bearing mature oothecae are dispersed due to an insecticide treatment, they have more opportunity to find a situation (harborage) favorable to oothecal hatch and survival of newly-hatched nymphs. When immature oothecae are retained, the likelihood of their maturing successfully is enhanced.

The only oothecae that could be distinguished on an individual basis as being dropped prematurely were those aborted during the exposure period. Although only seven were dropped, mortality of their parent females was higher than that of females that retained oothecae—a finding that can be attributed to the association of premature drop with suceptibility noted above.

Many of the nymphs that emerged on diazinon or malathion treated surfaces were unable to shed the embryonic cuticle–a phenomenon that was reported previously following exposure to a variety of insecticides (Lawson 1949, Killough 1958, Harmon and Ross 1987). The present study supports Killough (1958) who concluded that entanglement would not occur unless an insecticide came in contact with nymphs during emergence. Entanglement was nearly complete in the susceptible strain but not the malathion or diazinon resistant strains. On the basis that entanglement would eventually have resulted in death, mortality of susceptible strains nymphs exceeded that of the resistant nymphs. The frequency of susceptible nymphs that were dead at the time of observation (24 h after hatch) did not differ markedly from that of resistant strain nymphs, although a tendency towards higher mortality of susceptible nymphs occurred in the malathion experiment and, in the diazinon experiment, the difference was significant.

Whether drop would be sufficiently rapid to cause oothecae to fall on a treated surface would depend on both resistance characteristics of the target population and on the choice of insecticides. If oothecae did happen to hatch on a treated surface, the effects of malathion or diazinon on resistant strain nymphs would probably be less severe than those of propoxur (Harmon and Ross 1987). At least some of the nymphs that survived for 24 h on the treated surfaces were free of the embryonic cuticle and could have left the treated surface if permitted to do so. Nymphs that were still alive after a similar exposure to propoxur were either entangled in the cuticle or were in the knockdown condition. We conclude that oothecal retention by resistant females, as well as decreased mortality, adds to the difficulty of controlling insecticide resistant populations of the German cockroach.

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